

intramolecular to an intermolecular triple-stranded coiled-coil structure.
AUTHOR: Zuo J; Baler R; Dahl G; Voellmy R
CORPORATE SOURCE: Department of Biochemistry, University of Miami School of Medicine, Florida 33101.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1994 Nov) 14 (11) 7557-68.
PUB. COUNTRY: United States
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
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Entered Medline: 19941118

AB Heat stress regulation of human heat shock genes is mediated by human heat shock transcription factor hHSF1, which contains three 4-3 hydrophobic repeats (L21 to L23). In unstressed human cells (37 degrees C), hHSF1 appears to be in an inactive, monomeric state that may be maintained through intramolecular interactions stabilized by transient interaction with hsp70. Heat stress (39 to 42 degrees C) disrupts these interactions, and hHSF1 homotrimerizes and acquires heat shock element DNA-binding ability. hHSF1 expressed in Xenopus oocytes also assumes a monomeric, non-DNA-binding state and is converted to a trimeric, DNA-binding form upon exposure of the oocytes to heat shock (35 to 37 degrees C in this organism). Because endogenous HSF DNA-binding activity is low and anti-hHSF1 antibody does not recognize Xenopus HSF, we employed this system for mapping regions in hHSF1 that are required for the maintenance of the monomeric state. The results of mutagenesis analyses strongly suggest that the inactive hHSF1 monomer is stabilized by hydrophobic interactions involving all three leucine zippers which may form a triple-stranded coiled coil. Trimerization may enable the DNA-binding function of hHSF1 by facilitating cooperative binding of monomeric DNA-binding domains to the heat shock element motif. This view is supported by observations that several different LexA DNA-binding domain-hHSF1 chimeras bind to a LexA-binding site in a heat-regulated fashion, that single amino acid replacements disrupting the integrity of hydrophobic repeats render these chimeras constitutively trimeric and DNA binding, and that LexA itself binds stably to DNA only as a dimer but not as a monomer in our assays.

SO MOLECULAR AND CELLULAR BIOLOGY, (1994 Nov) 14 (11) 7557-68.
Journal code: NGY; 8109087. ISSN: 0270-7306.

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L4 ANSWER 3 OF 19 MEDLINE
DUPLICATE 3
ACCESSION NUMBER: 95094828 MEDLINE
DOCUMENT NUMBER: 95094828 PubMed ID: 8001584
TITLE: Isolation of a novel Plasmodium falciparum gene encoding a protein homologous to the Tat-binding protein family.
AUTHOR: Hirtzelin J; Farber P M; Franklin R M
CORPORATE SOURCE: Department of Structural Biology, University of Basel, Switzerland.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1994 Dec 1) 226 (2) 673-80.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X77914
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19950215
Entered Medline: 19950126

AB We have cloned a Plasmodium falciparum gene that belongs to the nuclear Tat-binding protein (TBP) gene family. This gene, PfTBP, is (A + T)-rich and encodes a 49.5-kDa protein. The predicted protein encoded by this gene has highest similarity to the slime mold protein DdTBP10 (86%) and to the yeast protein SUG1 (81.8%), both of which belong to the Tat-binding protein family. In agreement with the characteristics of this family, PfTBP contains highly conserved domain of approximately 200 amino acids, in which are found the motifs A and B of ATPases, and amino acid sequences characteristic of a large family of RNA or DNA helicases, suggesting a role in RNA or DNA unwinding. Like DdTBP10, the PfTBP protein has a heptad repeat of four leucine residues, reminiscent of a leucine zipper motif known to mediate dimerization. We have further characterized PfTBP gene expression by Northern-blot analysis. This gene is expressed in a stage-specific manner, with higher expression in the late trophozoite stage. The recombinant PfTBP gene has been expressed in Escherichia coli and a polyclonal antiserum has been raised in rabbits against the recombinant protein. This antibody has been used to study the protein in the parasite. The gene product is expressed in a stage-specific manner with higher expression in the late trophozoite and schizont stages, and is localized in the nucleus of the erythrocytic stage parasite. Thus the protein might have a function in DNA synthesis and/or in transcription, as is the case for other Tat-binding proteins.

SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1994 Dec 1) 226 (2) 673-80.
Journal code: EMZ; 0107600. ISSN: 0014-2956.

AB . . . RNA or DNA unwinding. Like DdTBP10, the PfTBP protein has a heptad repeat of four leucine residues, reminiscent of a leucine zipper motif known to mediate dimerization. We have further characterized PfTBP gene expression by Northern-blot analysis. This gene is expressed in a stage-specific manner, with higher. . . . has been expressed in Escherichia coli and a polyclonal antiserum has been raised in rabbits against the recombinant protein. This antibody has been used to study the protein in the parasite. The gene product is expressed in a stage-specific manner with. . . .

L4 ANSWER 4 OF 19 MEDLINE
DUPLICATE 4
ACCESSION NUMBER: 95018232 MEDLINE

DOCUMENT NUMBER: 95018232 PubMed ID: 7518664
TITLE: Correctly folded T-cell receptor fragments in the periplasm of *Escherichia coli*. Influence of folding catalysts.
AUTHOR: Wulfing C; Pluckthun A
CORPORATE SOURCE: Max-Planck-Institut für Biochemie, Protein Engineering Group, Martinsried, Germany.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1994 Oct 7) 242 (5) 655-69.
Journal code: JMB; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19941222
Entered Medline: 19941027

AB The T-cell receptor is the central recognition molecule in cellular immunity. Its extracellular domains are homologous with and thought to be structurally similar to an antibody Fab fragment. Despite the biological importance of the TCR and the ease of bacterial expression of antibody fragments, there are only few reports of TCR-fragment expression in *E. coli*. In order to understand the difficulties of expressing correctly folded TCR fragments in *E. coli*, we have characterized the expression behavior of single-chain Fv analogs of three different TCRs (scTCR). All of them can be folded into the correct conformation in the periplasm of *E. coli*, yet the extent of correct folding varies greatly. In order to overcome the folding problems of some of the scTCRs, we have developed a system with enhanced in vivo folding capability based on the simultaneous induction of the heat-shock response and over-expression of the *E. coli* disulfide isomerase DsbA at low temperature. We present a model describing the folding of the scTCRs in the periplasm of *E. coli* and possible points of folding assistance. The role of the periplasm as an independent folding compartment is emphasized and the existence of a general periplasmic chaperone is postulated. We have also shown that a bivalent scTCR, dimerized in vivo with helix-turn-helix modules, can be expressed in a correctly folded form.

SO JOURNAL OF MOLECULAR BIOLOGY, (1994 Oct 7) 242 (5) 655-69.
Journal code: JMB; 2985088R. ISSN: 0022-2836.

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L4 ANSWER 5 OF 19 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 94312012 MEDLINE
DOCUMENT NUMBER: 94312012 PubMed ID: 7518664
TITLE: Identification of antigenic regions of the human S2kD Ro/SS-A protein recognized by patient sera.
AUTHOR: Blangsted I; Ringertz N R; Pettersson I
CORPORATE SOURCE: Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden.
SOURCE: JOURNAL OF AUTOIMMUNITY, (1994 Apr) 7 (2) 263-74.
Journal code: ADL; 8812164. ISSN: 0896-8411.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940905
Last Updated on STN: 19960129
Entered Medline: 19940823

AB Patients with several different connective tissue diseases including Sjögren's syndrome and systemic lupus erythematosus produce autoantibodies reacting with a 52kD protein component of the Ro/SS-A antigen. Antibody recognition of recombinant Ro 52kD proteins encoded by both full-length and deletion clones was analysed by immunoblotting with patient sera. An antigenic region recognized by all anti-Ro 52kD positive sera was found in the middle part of the protein. By further mapping of residues 136-292 with overlapping clones, at least two independent epitopes within the domain were detected. This part of the protein contains a leucine zipper motif and shows structural similarities with a predicted coiled-coil region involved in protein dimer formation. In addition, one fifth of the sera reacted weakly with another antigenic region located in the amino-terminal part of the protein.

SO JOURNAL OF AUTOIMMUNITY, (1994 Apr) 7 (2) 263-74.
Journal code: ADL; 8812164. ISSN: 0896-8411.

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L4 ANSWER 6 OF 19 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 94030934 MEDLINE
DOCUMENT NUMBER: 94030934 PubMed ID: 7764189
TITLE: Improved bivalent miniantibodies, with identical avidity as whole antibodies, produced by high cell density fermentation of *Escherichia coli*.
AUTHOR: Pack P; Kujau M; Schroeckel V; Knupfer U; Wenderoth R;
Riesenbeck D; Pluckthun A
CORPORATE SOURCE: Max-Planck-Institut für Biochemie, Protein Engineering Group, Martinsried, Fed. Rep. Germany.
SOURCE: BIOTECHNOLOGY, (1993 Nov) 11 (11) 1271-7.
Journal code: ALI; 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE:

English

FILE SEGMENT:

B

ENTRY MONTH:

199312

ENTRY DATE:

Entered STN: 19950809

Last Updated on STN: 19950809

Entered Medline: 19931220

AB The combination of single-chain Fv-fragments (scFv) with a C-terminal, flexible linking region followed by a designed or natural dimerization domain provides a versatile system for targeted association of functional fragments in the periplasmic space of Escherichia coli. For homodimerization in vivo, two scFv fragments with a C-terminal hinge followed by a helix-turn-helix motif form "miniantibodies" with significantly higher avidity than in the case of leucine zipper containing constructs. The favorable design probably results in an antiparallel four-helix bundle and brings the homodimer to the same avidity as the whole IgA antibody, from which the binding site was taken. The molecular weight of the bivalent miniantibody is almost the same as that of a monovalent Fab fragment. We report here a high-cell density fermentation of E. coli producing these miniantibodies and a work-up procedure suitable for large scale production. Without any need of subsequent chemical coupling in vitro, approximately 200 mg/l of functional dimeric miniantibodies can be directly obtained from the E. coli culture.

SO BIO/TECHNOLOGY, (1993 Nov) 11 (11) 1271-7.

Journal code: ALL; 8309273, ISSN: 0733-222X.

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L4 ANSWER 7 OF 19 MEDLINE

DUPLICATE 7

ACCESSION NUMBER: 93107070 MEDLINE

DOCUMENT NUMBER: 93107070 PubMed ID: 7678004

TITLE: Zipper protein, a newly described tropomyosin-like protein of the intestinal brush border.

AUTHOR: Bikle D D; Munson S; Morrison N; Eiseman J
CORPORATE SOURCE: Department of Medicine, University of California, San Francisco.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Jan 5) 268 (1) 620-6.

JOURNAL CODE: HIV; 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

JOURNAL; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-L02620; GENBANK-L02621; GENBANK-L02622

ENTRY MONTH: 199301

ENTRY DATE: Entered STN: 19930212

Last Updated on STN: 19960129

Entered Medline: 19930128

AB We have cloned and sequenced from a chick intestinal library the cDNA for a new tropomyosin-like protein with an extensive leucine zipper motif. The cDNA recognized a 2.5-kilobase transcript with highest levels in the intestine. The open reading frame encoded a protein with 239 residues (28 kDa), the deduced sequence of which forms 27 heptad repeats, 21 of which begin with leucine and the other 6 with conservative substitutions (methionine, valine, threonine). This sequence predicts a coiled coil dimer similar to that of tropomyosin with which it has 34% homology. We have named this newly described protein zipper protein. The protein was expressed in bacteria. Antibodies were made to peptides representing different regions of the deduced sequence and tested for their ability to recognize the recombinant zipper protein on immunoblots. Such antibodies were used to immunolocalize zipper protein to the intestinal brush border. A radioimmunoassay was then established using recombinant zipper protein as standard and tracer and one of the affinity-purified antisera as primary antibody. Extracts from intestine, kidney, and liver displaced tracer zipper protein in parallel with that of the standard curve. A radioimmunoassay was then established using recombinant zipper protein as standard and tracer and one of the affinity-purified antisera as primary antibody. Extracts from intestine, kidney, and liver displaced tracer zipper protein in parallel with that of the standard curve, and zipper. . .

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Jan 5) 268 (1) 620-6.

Journal code: HIV; 2985121R. ISSN: 0021-9258.

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L4 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:583901 CAPLUS

DOCUMENT NUMBER: 123:30498

TITLE: The N-myc-Max(p20/22) nucleoprotein complex in human neuroblastoma cells
AUTHOR(S): Wenzel, Achim; Cziepluch, Celina; Hamann, Ute;
Schuermann, Joerg; Schwab, Manfred
CORPORATE SOURCE: Department Cytogenetics, German Cancer Research Center, Heidelberg, 6900, Germany
SOURCE: Hum. Neuroblastoma (1993), 105-17.
Editor(s): Schwab, Manfred; Tonini, Gian Paolo;
Benard, Jean. Harwood: Chur, Switz.
CODEN: 61FSAQ

DOCUMENT TYPE: Conference
LANGUAGE: English

AB Due to amplification of the N-myc gene the N-Myc oncprotein is overexpressed in certain human tumors that share neuroectodermal features. The proteins encoded by the proto-oncogenes c-myc, L-myc, and N-myc contain at their C-terminus a tripartite segment comprising a basic DNA-binding region (BR), a helix-loop-helix (HLH) and a leucine zipper motif (Zip), that are thought to be involved in DNA binding and protein-protein oligomerization. The authors identified in exts. of human neuroblastoma cells two nuclear phosphoproteins p20/22 forming a hetero-oligomeric complex with N-Myc by immunopptn. using a monoclonal anti-N-Myc antibody. Both proteins are structurally related and p20 is identical with Max, a recently identified binding partner of myc-proteins. Max(p20/22) is a target of phosphorylation by casein kinase II (CK-II) in vitro. Dimerization of N-Myc with Max(p20/22) occurs via the HLH-Zip region of the N-Myc oncprotein.

SO Hum. Neuroblastoma (1993), 105-17. Editor(s): Schwab, Manfred; Tonini, Gian Paolo; Benard, Jean. Publisher: Harwood, Chur, Switz.
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L4 ANSWER 9 OF 19 MEDLINE DUPLICATE 8

ACCESSION NUMBER: 94109697 MEDLINE
DOCUMENT NUMBER: 94109697 PubMed ID: 8282202
TITLE: Display of biologically active proteins on the surface of filamentous phages: a cDNA cloning system for selection of functional gene products linked to the genetic information responsible for their production.

AUTHOR: Cramer R; Suter M
CORPORATE SOURCE: Swiss Institute of Allergy and Asthma Research, Davos.
SOURCE: GENE, (1993 Dec 27) 137 (1) 69-75.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940228
Last Updated on STN: 19940228
Entered Medline: 19940217

AB A cloning and expression system allowing display of functional cDNAs or other gene products on the surface of filamentous phage has been developed, exploiting the high-affinity interaction of the Jun and Fos leucine zippers. Gene jun was expressed from a lacZ promoter as a fusion protein with the viral coat protein, pIII, thereby being structurally incorporated into phage particles during infection with a helper phage. Using a second lacZ promoter of the phagemid, gene fos was co-expressed as an N-terminal fusion peptide to cDNA library gene products, so that the resulting Fos-fusion proteins could become associated with the Jun-decorated phage particles. To avoid interphage exchange of fos-cDNA fusion products, cysteines were engineered at the N- and C-termini of each of the leucine zippers, providing a covalent link of the cDNA gene product to the genetic instructions required for its production. Dissociation between phage and cDNA gene products was readily achieved using reducing agents. Phages displaying gene products covalently anchored on their surface via the modified leucine zippers can be selectively enriched 10⁽⁴⁾-10⁽⁶⁾-fold over nonspecific phages using antibodies. Thus, this cloning system allows rapid isolation of rare mRNA products from complex cDNA libraries by enrichment with appropriate ligands. This approach should allow the expression and cloning of dimeric proteins by cDNA shuffling.

SO GENE, (1993 Dec 27) 137 (1) 69-75.
Journal code: FOP; 7706761. ISSN: 0378-1119.

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L4 ANSWER 10 OF 19 MEDLINE DUPLICATE 9

ACCESSION NUMBER: 92348423 MEDLINE
DOCUMENT NUMBER: 92348423 PubMed ID: 1639803
TITLE: Subunit structure of cell-specific E box-binding proteins analyzed by quantitation of electrophoretic mobility shift.
AUTHOR: Park C W; Walker M D
CORPORATE SOURCE: Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Aug 5) 267 (22) 15642-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920911
Last Updated on STN: 19920911
Entered Medline: 19920828

AB Expression of insulin and immunoglobulin genes is dependent on the presence of E boxes (consensus sequence CAXXTG) within the enhancer regions. These sequences are recognized by cell-specific nuclear factors IEF1 (insulin enhancer factor 1) and LEF1 (lymphoid enhancer factor 1). Although IEF1 and LEF1 are distinct by several parameters, they are both recognized by antisera to the mouse helix-loop-helix (HLH) protein A1 (a homolog of the human protein E47, product of the E2A gene). This suggests that A1/E47 or a close relative is a component of both complexes. In order to further characterize the complexes, we have used *in vitro* translated DNA-binding proteins of known size to verify that electrophoretic mobility shift analysis can be used to estimate the molecular weight of DNA-binding proteins from both the HLH family and the leucine zipper family. Under the conditions used, migration is relatively insensitive to changes in protein charge. This analysis, in combination with mixing experiments between nuclear extracts and *in vitro* translated HLH proteins, indicates that IEF1 and LEF1 are dimeric complexes. IEF1 behaves as a complex of two proteins, one of which is 67 kDa and is recognized by antibodies to A1, and the second of which is 25 kDa. LEF1 on the other hand, appears to be a complex of two proteins of 67 kDa. The size of the 67-kDa subunits is consistent with that reported for the full-length E2A gene products. The 25-kDa subunit of IEF1 forms DNA-binding heterodimers with A1 but not MyoD and is present in a limited range of cell types, features characteristic of class B HLH proteins such as MyoD and achaete-scute. Taken together, the data support the idea that the E2A gene products are involved directly in regulation of insulin and immunoglobulin gene expression; regulation of the insulin gene apparently requires, in addition, the 25-kDa HLH protein (designated IESF1 for insulin enhancer-specific factor 1).

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Aug 5) 267 (22) 15642-9.
Journal code: HIV; 2985121R. ISSN: 0021-9258.

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L4 ANSWER 11 OF 19 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 92332476 MEDLINE
DOCUMENT NUMBER: 92332476 PubMed ID: 1629185
TITLE: Deletion of lacrose repressor carboxyl-terminal domain affects tetramer formation.
AUTHOR: Chen J; Matthews S
CORPORATE SOURCE: Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77251.
CONTRACT NUMBER: GM 22441 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Jul 15) 267 (20) 13843-50.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920904
Last Updated on STN: 19970203
Entered Medline: 19920814

AB The carboxyl-terminal sequence of the lac repressor protein contains heptad repeats of leucines at positions 342, 349, and 356 that are required for tetramer assembly, as substitution of these leucine residues yields solely dimeric species (Chakerian, A. E., Tesmer, V. M., Manly, S. P., Brackett, J. K., Lynch, M. J., Hoh, J. T., and Matthews, K. S. (1991) *J. Biol. Chem.* 266, 1371-1374; Alberti, S., Oehlert, S., von Wilcken-Bergmann, B., Kramer, H., and Muller-Hill, B. (1991) *New Biol.* 3, 57-62). To further investigate this region, which may form a leucine zipper motif, a family of lac repressor carboxyl-terminal deletion mutants eliminating the last 4, 5, 11, 18, and 32 amino acids (aa) has been constructed. The -4 aa mutant, in which all of the leucines in the presumed leucine zipper are intact, is tetrameric and displays operator and inducer binding properties similar to wild-type repressor. The -5 aa, -11 aa, -18 aa, and -32 aa deletion mutants, depleted of 1, 2, or all 3 of the leucines in the heptad repeats, are all dimeric, as demonstrated by gel filtration chromatography. Circular dichroism spectra and protease digestion studies indicate similar secondary/tertiary structures for the mutant and wild-type proteins. Differences in reaction with a monoclonal antibody specific for a subunit interface are observed for the dimeric versus tetrameric proteins, indicative of exposure of the target epitope as a consequence of deletion. Inducer binding properties of the deletion mutants are similar to wild-type tetrameric repressor at neutral pH. Only small differences in affinity and cooperativity from wild-type are evident at elevated pH; thus, the cooperative unit within the tetramer appears to be the dimer. "Apparent" operator binding affinity for the dimeric proteins is diminished, although minimal change in operator dissociation rate constants was observed. The diminution in apparent operator affinity may therefore derive from either 1) dissociation of the dimeric mutants to monomer generating a linked equilibrium or 2) alterations in intrinsic operator affinity of the dimers; the former explanation is favored. This detailed characterization of the purified mutant proteins confirms that the carboxyl-terminal region is involved in the

dimer-dimer interface and demonstrates cooperativity for inducer binding is contained within the dimer unit of the tetramer structure.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Jul 15) 267 (20) 13843-50.
Journal code: HIV; 2985121R. ISSN: 0021-9258.

AB . . . at positions 342, 349, and 356 that are required for tetramer assembly, as substitution of these leucine residues yields solely dimeric species (Chakerian, A. E., Tesmer, V. M., Manly, S. P., Brackett, J. K., Lynch, M. J., Hoh, J. T., and . . . B., Kramer, H., and Muller-Hill, B. (1991) New Biol. 3, 57-62). To further investigate this region, which may form a leucine zipper motif, a family of lac repressor carboxyl-terminal deletion mutants eliminating the last 4, 5, 11, 18, and 32 amino acids (aa) has been constructed. The -4 aa mutant, in which all of the leucines in the presumed leucine zipper are intact, is tetrameric and displays operator and inducer binding properties similar to wild-type repressor. The -5 aa, -11 aa, . . . -32 aa deletion mutants, depleted of 1, 2, or all 3 of the leucines in the heptad repeats, are all dimeric, as demonstrated by gel filtration chromatography. Circular dichroism spectra and protease digestion studies indicate similar secondary/tertiary structures for the mutant and wild-type proteins. Differences in reaction with a monoclonal antibody specific for a subunit interface are observed for the dimeric versus tetrameric proteins, indicative of exposure of the target epitope as a consequence of deletion. Inducer binding properties of the . . . and cooperativity from wild-type are evident at elevated pH; thus, the cooperative unit within the tetramer appears to be the dimer. "Apparent" operator binding affinity for the dimeric proteins is diminished, although minimal change in operator dissociation rate constants was observed. The diminution in apparent operator affinity may therefore derive from either 1) dissociation of the dimeric mutants to monomer generating a linked equilibrium or 2) alterations in intrinsic operator affinity of the dimers; the former explanation is favored. This detailed characterization of the purified mutant proteins confirms that the carboxyl-terminal region is involved in the dimer-dimer interface and demonstrates that cooperativity for inducer binding is contained within the dimer unit of the tetramer structure.

L4 ANSWER 12 OF 19 MEDLINE
DUPLICATE 11
ACCESSION NUMBER: 92262413 MEDLINE
DOCUMENT NUMBER: 92262413 PubMed ID: 1584756
TITLE: Alternative usage of initiation codons in mRNA encoding the cAMP-responsive-element modulator generates regulators with opposite functions.
AUTHOR: Delmas V; Laoide B M; Masquelier D; de Groot R P; Foulkes N S; Sascone-Corsi P
CORPORATE SOURCE: Laboratoire De Genetique Moleculaire des Eucaryotes, U184 Institut National de la Sante et de la Recherche Medicale, Faculte de Medecine, Strasbourg, France.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 May 15) 89 (10) 4226-30.
PUB. COUNTRY: Journal code: PV3; 7505876. ISSN: 0027-8424.
United States
LANGUAGE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: English
Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920626
Last Updated on STN: 19980206
Entered Medline: 19920616

AB The cAMP-responsive-element modulator (CREM) gene encodes both antagonists (CREM alpha/beta/gamma) and an activator (CREM tau) of cAMP-responsive transcription by alternative splicing. In adult mouse brain a predominant 21-kDa protein, not corresponding to any previously characterized transcript, is detected with specific CREM antibodies. A developmental switch occurs in brain as expression changes at birth from CREM alpha/beta to the 21-kDa protein. We show that the 21-kDa protein corresponds to S-CREM (short CREM), a protein produced by the use of an internal AUG initiation codon in the CREM tau transcript. S-CREM shares with the other CREM proteins the basic DNA-binding and leucine-zipper dimerization domain. S-CREM functions as a transcriptional repressor of cAMP-induced transcription. Thus, two proteins with opposite functions are generated by alternative translation using two AUG codons within the same reading frame.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 May 15) 89 (10) 4226-30.
Journal code: PV3; 7505876. ISSN: 0027-8424.

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L4 ANSWER 13 OF 19 MEDLINE
DUPLICATE 12
ACCESSION NUMBER: 92144568 MEDLINE
DOCUMENT NUMBER: 92144568 PubMed ID: 1737014
TITLE: Miniantibodies: use of amphiphilic helices to produce functional, flexibly linked dimeric Fv fragments with high avidity in Escherichia coli.
AUTHOR: Pack P; Pluckthun A
CORPORATE SOURCE: Genzentrum Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, FRG.
SOURCE: BIOCHEMISTRY, (1992 Feb 18) 31 (6) 1579-84.
Journal code: AOG; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
LANGUAGE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: English
Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920405
Last Updated on STN: 19920405
Entered Medline: 19920317

AB We have designed dimeric antibody fragments that assemble in Escherichia coli. They are based on single-chain Fv fragments, with a flexible hinge region from mouse IgG3 and an amphiphilic helix

fused to the C-terminus of the antibody fragment. The sequence of the helix was taken either from that of a previously reported four-helix bundle design or from a leucine zipper, optionally extended with a short cysteine-containing peptide. The bivalent fragments associate *in vivo*, either with covalent linkage or with a monomer-dimer equilibrium, and results from ultracentrifugation sedimentation studies and SDS-PAGE are consistent with dimers. All constructs are able to bind to surface-bound antigen under conditions in which only bivalent but not monovalent antibody fragments bind. The covalent bundle helix construct shows binding characteristics nearly identical to those of the much larger whole mouse antibody, resulting in substantially more stable immunoglobulin-antigen complexes than in the case of monovalent fragments. This modular design of natural and engineered protein domains directly leads to a boost of avidity, and it allows the construction of bispecific antibody fragments in functional form in *E. coli*.

SO BIOCHEMISTRY, (1992 Feb 18) 31 (6) 1579-84.

Journal code: A0G; 0370623. ISSN: 0006-2960.

AB We have designed dimeric antibody fragments that assemble in *Escherichia coli*. They are based on single-chain FV fragments, with a flexible hinge region from mouse IgG3 and an amphiphilic helix fused to the C-terminus of the antibody fragment. The sequence of the helix was taken either from that of a previously reported four-helix bundle design or from a leucine zipper, optionally extended with a short cysteine-containing peptide. The bivalent fragments associate *in vivo*, either with covalent linkage or with a monomer-dimer equilibrium, and results from ultracentrifugation sedimentation studies and SDS-PAGE are consistent with dimers. All constructs are able to bind to surface-bound antigen under conditions in which only bivalent but not monovalent antibody fragments bind. The covalent bundle helix construct shows binding characteristics nearly identical to those of the much larger whole mouse antibody, resulting in substantially more stable immunoglobulin-antigen complexes than in the case of monovalent fragments. This modular design of natural and engineered protein domains directly leads to a boost of avidity, and it allows the construction of bispecific antibody fragments in functional form in *E. coli*.

L4 ANSWER 14 OF 19 MEDLINE

DUPLICATE 13

ACCESSION NUMBER: 92271211 MEDLINE
 DOCUMENT NUMBER: 92271211 PubMed ID: 1589769
 TITLE: Interaction cloning: identification of a helix-loop-helix zipper protein that interacts with c-Fos.
 AUTHOR: Blanar M A; Rutter W J
 CORPORATE SOURCE: Hormone Research Institute, University of California, San Francisco 94143.
 CONTRACT NUMBER: DK-21344 (NIDDK)
 DK-41822 (NIDDK)
 SOURCE: SCIENCE, (1992 May 15) 256 (5059) 1014-8.
 Journal code: UJ7; 0404511. ISSN: 0036-8075.
 PUB. COUNTRY: United States
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 OTHER SOURCE: GENBANK-M77476
 ENTRY MONTH: 199206
 ENTRY DATE: Entered STN: 19920710
 Last Updated on STN: 19970203
 Entered Medline: 19920619

AB A facile method for isolating genes that encode interacting proteins has been developed with a polypeptide probe that contains an amino-terminal extension with recognition sites for a monoclonal antibody, a specific endopeptidase, and a site-specific protein kinase. This probe, containing the basic region-leucine zipper dimerization motif of c-Fos, was used to screen a complementary DNA library. A complementary DNA that encoded a member of the basic-helix-loop-helix-zipper (bHLH-Zip) family of proteins was isolated. The complementary DNA-encoded polypeptide FIP (Fos interacting protein) bound to oligonucleotide probes that contained DNA binding motifs for other HLH proteins. When cotransfected with c-Fos, FIP stimulated transcription of an AP-1-responsive promoter.

SCIENCE, (1992 May 15) 256 (5059) 1014-8.
 Journal code: UJ7; 0404511. ISSN: 0036-8075.

AB . . . interacting proteins has been developed with a polypeptide probe that contains an amino-terminal extension with recognition sites for a monoclonal antibody, a specific endopeptidase, and a site-specific protein kinase. This probe, containing the basic region-leucine zipper dimerization motif of c-Fos, was used to screen a complementary DNA library. A complementary DNA that encoded a member of the . . .

L4 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1992:646102 CAPLUS
 DOCUMENT NUMBER: 117:246102
 TITLE: Basement membrane genes and transcription factors
 AUTHOR(S): Burbelo, Peter; Gabriel, Gary; Wujeck, J.; Kedar, Vishram V.; Weeks, Benjamin S.; Kleinman, Hynda K.; Yamada, Yoshihiko
 CORPORATE SOURCE: Lab. Dev. Biol., Natl. Inst. Dent. Res., Bethesda, MD, USA
 SOURCE: Colloq. INSERM (1992), 216(Cell Mol. Aspects Cirrhosis), 135-45
 DOCUMENT TYPE: CODEN: CINMDE; ISSN: 0768-3154
 LANGUAGE: Journal; General Review
 English

AB A review with 23 refs. Basement membrane gene expression is of crit. importance in cell differentiation, growth and development. The 5'-end of the laminin B1 and B2 gene and of the .alpha.1 and .alpha.2 (IV) collagen genes have been isolated in order to understand the regulation of these genes. Using transfection anal., DNA footprinting and gel shift assays, several DNA regulatory elements have been identified. Work in progress will elucidate the transcription factors which bind to these regulatory elements. Laminin induces differentiation, but the exact mechanisms are unclear. Using a differential screen, a transcription factor induced by laminin, Lilzip-1, has been identified in neural cells. A complete Lilzip-1 cDNA has been obtained and characterized as having a serine-rich domain, a highly basic DNA-binding domain and a leucine zipper dimerization domain. Anti-lilzip antibodies detected a 46 kDa protein in tissues rich in basement membrane such as brain, kidney, and lung. DNA binding expts. indicate Lilzip has a high affinity for AP-1 and CRE/ATF DNA sequences. Thus, laminin-induced differentiation may involve the induction of specific

- transcription factors.
- SO Colloq. INSERM (1992), 216(Cell Mol. Aspects Cirrhosis), 135-45
CODEN: CINMDE; ISSN: 0768-3154
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- L4 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:166991 CAPLUS
DOCUMENT NUMBER: 118:166991
TITLE: Mono- and bivalent antibody fragments produced in E. coli: binding properties and folding in vivo
AUTHOR(S): Pack, Peter; Knappik, Achim; Krebber, Claus;
Plueckthun, Andreas
CORPORATE SOURCE: Max-Planck-Inst. Biochem., Martinsried, D-8033, Germany
SOURCE: Harnessing Biotechnol. 21st Century, Proc. Int. Biotechnol. Symp. Expo., 9th (1992), 10-13.
Editor(s): Ladisch, Michael R.; Bose, Arindam. ACS: Washington, D.C.
CODEN: 58ODAU
DOCUMENT TYPE: Conference
LANGUAGE: English
- AB Dimeric antibody fragments of minimal size that assemble in Escherichia coli and show an increase in avidity approaching a whole antibody were designed. They are based on single-chain Fv fragments with a flexible hinge region from mouse IgG3 and an amphiphilic helix fused to the C-terminus of the antibody fragment. The sequence of the helix was taken either from that of a 4-helix bundle design or a leucine zipper, optionally extended with a short cysteine contg. peptide. To investigate the folding and assembly process of antibody fragment in E. coli, co-expression expts. with proline cis-trans-isomerase and disulfide isomerase were carried out. These folding steps do not appear to be limiting the folding process in E. coli.
- SO Harnessing Biotechnol. 21st Century, Proc. Int. Biotechnol. Symp. Expo., 9th (1992), 10-13. Editor(s): Ladisch, Michael R.; Bose, Arindam. Publisher: ACS, Washington, D.C.
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- L4 ANSWER 17 OF 19 MEDLINE
ACCESSION NUMBER: 91257572 MEDLINE
DOCUMENT NUMBER: 91257572 PubMed ID: 2044953
TITLE: The leucine zipper of TFE3 dictates helix-loop-helix dimerization specificity.
AUTHOR: Beckmann H; Kadesch T
CORPORATE SOURCE: Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Philadelphia 19104-6148.
SOURCE: GENES AND DEVELOPMENT, (1991 Jun) 5 (6) 1057-66.
Journal code: FN3; 8711660. ISSN: 0890-9369.
PUB. COUNTRY: United States
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910802
Last Updated on STN: 19910802
Entered Medline: 19910712
- AB TFE3 is a DNA-binding protein that activates transcription through the muE3 site of the immunoglobulin heavy-chain enhancer. Its amino acid sequence reveals two putative protein dimerization motifs: a helix-loop-helix (HLH) and an adjacent leucine zipper. We show here that both of these motifs are necessary for TFE3 to homodimerize and to bind DNA in vitro. Using a dominant negative TFE3 mutant, we also demonstrate that both the HLH and the leucine zipper motifs are necessary and sufficient for protein-protein interactions in vivo. TFE3 is unable to form stable heterodimers with a variety of other HLH proteins, including USF, a protein that is structurally similar to TFE3 and binds a common DNA sequence. The analysis of "zipper swap" proteins in which the TFE3 HLH was fused to the leucine zipper region of USF indicates that dimerization specificity is mediated entirely by the identity of the leucine zipper and its position relative to the HLH. Hence, in this "b-HLH-zip" class of proteins, the leucine zipper functions in concert with the HLH both to stabilize protein-protein interactions and to establish dimerization specificity.
- SO GENES AND DEVELOPMENT, (1991 Jun) 5 (6) 1057-66.
Journal code: FN3; 8711660. ISSN: 0890-9369.
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mutant, we also demonstrate that both the HLH and the leucine zipper motifs are necessary and sufficient for protein-protein interactions in vivo. TFE3 is unable to form stable heterodimers with a variety. . . binds a common DNA sequence. The analysis of "zipper swap" proteins in which the TFE3 HLH was fused to the leucine zipper region of USF indicates that dimerization specificity is mediated entirely by the identity of the leucine zipper and its position relative to the HLH. Hence, in this "b-HLH-zip" class of proteins, the leucine zipper functions in concert with the HLH both to stabilize protein-protein interactions and to establish dimerization specificity.

L4 ANSWER 18 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:355391 BIOSIS
DOCUMENT NUMBER: BR41:39906
TITLE: AN EMPIRICAL APPROACH TO FREE ENERGY AND SPECIFICITY OF MACROMOLECULAR INTERACTIONS.
AUTHOR(S): NOVOTNY J; BEHLING R; BRUCCOLERI R E; KRYSTEK S
CORPORATE SOURCE: BRISTOL-MYERS-SQUIBB RES. INST., PRINCETON, N.J. 08543-4000.
SOURCE: MEETING ON PROTEIN FOLDING, STRUCTURE AND FUNCTION HELD AT THE 20TH ANNUAL MEETING OF THE KEYSTONE SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, KEYSTONE, COLORADO, USA, APRIL 8-14, 1991. J CELL BIOCHEM SUPPL, (1991) 0 (15 PART G), 167.
CODEN: JCBSD7.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English
SO MEETING ON PROTEIN FOLDING, STRUCTURE AND FUNCTION HELD AT THE 20TH ANNUAL MEETING OF THE KEYSTONE SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, KEYSTONE, COLORADO, USA, APRIL 8-14, 1991. J CELL BIOCHEM SUPPL. (1991) 0 (15 PART G), 167.
CODEN: JCBSD7.

IT Miscellaneous Descriptors

ABSTRACT ANTIGEN-ANTIBODY COMPLEXES ENZYME INHIBITOR
COMPLEXES LEUCINE ZIPPER DIMERS
DNA-OPERATOR COMPLEXES DNA-REPRESSOR COMPLEXES THERMODYNAMICS

L4 ANSWER 19 OF 19 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 92131053 MEDLINE
DOCUMENT NUMBER: 92131053 PubMed ID: 1775160
TITLE: Cloning and sequence analysis of the Schistosoma mansoni membrane glycoprotein antigen gene GP22.
AUTHOR: el-Sherbeini M; Ramadan N; Bostian K A; Knopf P M
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Merck, Sharp and Dohme Research Laboratories, Rahway, NJ 07065.
CONTRACT NUMBER: AI-02650 (NIAID)
AI-21380 (NIAID)
AI-31224 (NIAID)
SOURCE: MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1991 Nov) 49 (1) 83-98.
PUB. COUNTRY: Journal code: NOR; 8006324. ISSN: 0166-6851.
Netherlands
LANGUAGE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: English
Priority Journals
OTHER SOURCE: GENBANK-M34357; GENBANK-M63270; GENBANK-M63271;
GENBANK-M63272; GENBANK-M63273; GENBANK-M63274;
GENBANK-M63275; GENBANK-M63276; GENBANK-M63277;
GENBANK-M63278; GENBANK-S77745

ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920322
Last Updated on STN: 19920322
Entered Medline: 19920228

AB A family of Schistosoma mansoni proteins (18-22 kDa, pI 5.3-5.8) are biosynthesized in juvenile worms and immunoprecipitated by antibodies uniquely present in protective Fischer rat antiserum. A cDNA clone, lambda gtl1-40, expressing epitopes common to this protein family was used to obtain a genomic DNA clone, by hybridization with a lambda gtl1-40 oligonucleotide probe. In the 1.37 kb of genomic DNA sequenced, an open reading frame of 182 amino acids was identified on the strand corresponding to lambda gtl1-40 coding sequences, and those of identical independently isolated cDNA clones defining a 25-kDa surface membrane glycoprotein. The new S. mansoni gene is termed GF22. There are two candidate promoters, confirmed by primer extension studies with worm RNA. Promoter 1 (P1) is preceded by a G + C-rich region and potential CAAT sequences, and is to the 5'-side of P2. Transcription from P1 is initiated at 2 different sites, apparently producing mRNAs with different translation start sites (ATG). Decoding these mRNAs yields protein products of 182 (P1), 175 (P1), 140 (P2) and 136 (P2) amino acids. The polypeptides share the following features: a hydrophobic segment near the carboxy terminus sufficient to span a lipid bilayer, with a consensus sequence for thio-esterification by a fatty acid; an external domain containing 2 potential N-linked glycosylation sites; and a candidate leucine-zipper motif, suggesting the protein may exist as a dimer on the worm surface. While sharing these common features in their carboxy terminal regions, the three proteins differ in the length and properties of their amino termini. The 140-amino acid protein has a short hydrophobic amino terminus, while the 175- and 182-amino acid proteins have more extensive hydrophobic sequences, each preceded by a hydrophilic amino terminal sequence. The heterogeneity observed in 2-dimensional gels of the antigen may be explained in part by the size and charge differences among the proteins deduced from the sequence and transcription pattern of this gene. The possibility of stage-specific regulated expression of this candidate vaccine antigen family is an attractive concept, potentially accounting for the phenomenon of concomitant immunity observed in the rat and perhaps other schistosome hosts.

SO MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1991 Nov) 49 (1) 83-98.
Journal code: NOR; 8006324. ISSN: 0166-6851.

AB A family of Schistosoma mansoni proteins (18-22 kDa, pI 5.3-5.8) are biosynthesized in juvenile worms and immunoprecipitated by antibodies uniquely present in protective Fischer rat antiserum. A cDNA clone, lambda gtl1-40, expressing epitopes common to this protein family was. . . consensus sequence for thio-esterification by a fatty acid; an external domain containing 2 potential N-linked glycosylation sites; and a candidate leucine-zipper motif, suggesting the protein may exist as a dimer on the worm surface. While sharing these common features in their carboxy terminal regions, the three proteins differ in the